EXHIBIT 2

Sprue-like histology in patients with abdominal pain taking olmesartan compared with other angiotensin receptor blockers

Stephen M Lagana,[1] Eric D Braunstein,[2] Carolina Arguelles-Grande,[2] Govind Bhagat,[1] Peter H R Green,[2] Benjamin Lebwohl[2]

[1]Department of Pathology and Cell Biology, Columbia University and New York Presbyterian Hospital, New York, New York, USA
[2]Department of Medicine, Celiac Disease Center, Columbia University Medical Center, New York, New York, USA

Correspondence to
Dr Stephen M Lagana, Department of Pathology and Cell Biology, Columbia University and New York Presbyterian Hospital, 630 W 168th St., VC15-202, New York, NY 10032, USA; Sml2179@cumc.columbia.edu

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ABSTRACT

Aims A severe syndrome characterised by life-threatening diarrhoea and severe sprue-like histology has been described in patients taking the angiotensin receptor blocker (ARB) olmesartan. It is unknown whether there are any histopathological changes in patients without severe diarrhoea exposed to this medication. It is also unknown whether other ARBs cause sprue-like histology.

Methods Retrospective cohort study of patients with abdominal pain undergoing upper gastrointestinal endoscopy with duodenal biopsy who were taking ARBs. Patients taking olmesartan (n=20) and a non-olmesartan ARB (n=20) were compared with age and sex-matched controls. Histological features (classic sprue-like and other inflammatory changes) were analysed.

Results No single histopathological finding was significantly more common in olmesartan-using patients than controls. However, 10 of 20 olmesartan patients had one or more sprue-like histological features compared with 4 of 20 age-matched and sex-matched controls not taking ARBs (p=0.10). Patients taking ARBs other than olmesartan were not more likely than controls to have one or more of these sprue-like histological features (9/20 vs. 12/20, p=0.34).

Conclusions There were no statistically significant differences between olmesartan users with abdominal pain and controls for any single histopathological abnormality. However, there were trends towards significance for individual abnormalities as well as for a composite outcome of sprue-like changes. This raises the possibility that there is a spectrum of histological changes associated with olmesartan use.

INTRODUCTION

Olmesartan medoxomil is a commonly used antihypertensive medication, which acts by blocking angiotensin receptors. Recently, a series of cases were described in which 22 patients presented with debilitating diarrhoea and had a sprue-like enteropathy on histological examination due to olmesartan. The diarrhoea was so severe that 14 patients required hospitalisation and 4 required total parenteral nutrition. Serological testing for coeliac disease was negative in all cases and none improved with a gluten-free diet. All had biopsies, which showed severe sprue-like changes (villous atrophy, lamina propria inflammation and intraepithelial lymphocytosis (IEL)). Seven of the patients had collagenous sprue. All patients had dramatic improvement, with resolution of their diarrhoea following cessation of olmesartan.[1] As a major referral centre for coeliac disease, we have subsequently encountered a number of such cases and several other case series and reports have been published, which demonstrate similar clinical and histopathological findings.[2–12] At present, this adverse drug reaction is thought to be a rare occurrence. A recent case–control study did not show an association between olmesartan use and chronic diarrhoea in patients presenting for oesophagogastroduodenoscopy (OGD) or colonoscopy.[13]

While it is unusual to encounter severe villous atrophy in non-coeliac patients, milder changes which may overlap with sprue-like enteropathies (such mild or focal IEL) are common.[2 14] Medication reactions, particularly non-steroidal anti-inflammatory drugs, are commonly listed in the differential of such pathological findings.[15] Other drugs also enter the differential, but it is unknown whether olmesartan exposure should be considered when encountering such findings. It is also unknown whether other angiotensin receptor blockers (ARBs) may cause histopathological changes.

Because it is unclear whether the severe sprue-like enteropathy seen in a few patients taking olmesartan is the severe end of a spectrum of intestinal injury, we identified patients taking olmesartan who had undergone endoscopy for abdominal pain with duodenal biopsy and systematically studied the biopsies. We also identified patients with abdominal pain taking other ARBs who had duodenal biopsy and examined their biopsies to determine whether the changes were specific for olmesartan. We identified those patients whose indication for the procedure was abdominal pain to avoid those whose symptom was diarrhoea.

METHODS

We performed a retrospective cohort study using the electronic medical record of Columbia University Medical Center endoscopy unit (ProVation Medical Systems, Wolters Kluwer Health, New South Wales, Australia). This record includes all home medication use reported by outpatients undergoing OGD. This list of medications is ascertained by a trained nurse during an interview immediately preceding the procedure. We queried the medical record for patients in whom the indication for OGD was abdominal pain (self-reported, no formal diagnostic criteria employed) and identified 20 outpatients who listed olmesartan as one of their medications. We then matched each patient by age and gender to a control patient who did not report any ARB when listing his/her medications. Using the same process, we identified

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another 20 users of non-olmesartan ARBs and corresponding matched controls. We excluded all patients with a history of coeliac disease, inflammatory bowel disease or *Helicobacter pylori* infection (present or prior). In total, we identified 80 patients undergoing OGD for abdominal pain: 20 olmesartan users with 20 matched controls and 20 non-olmesartan ARB users with 20 matched controls. This study was approved by the Columbia University Medical Center Institutional Review Board.

Abnormalities that are seen in enteropathies that include coeliac disease and the sprue-like enteropathy of olmesartan including villous atrophy, crypt hyperplasia, increased IEL concentration, chronic lamina propria inflammation and increased subepithelial collagen deposition were evaluated on routine H&E-stained slides by a gastrointestinal pathologist who was blinded to the medication status (SML). The maximum IEL count in 100 epithelial cells was counted by routine H&E stain. In addition, increased crypt apoptosis (abnormal was considered more than 2 crypt apoptotic bodies in any 10 consecutive crypts or more than one apoptotic body per biopsy piece), active inflammation (defined as any extravascular neutrophils) and eosinophilia were also documented.

Statistical analysis

We compared the prevalence of each of the above histopathological findings among ARB users and their matched controls. We used the χ^2 and Fisher exact test when comparing proportions, and used the Mann–Whitney test when comparing IEL counts. After reviewing these comparisons, we subsequently performed a post-hoc analysis comparing ARB-exposed subjects with controls with regard to the composite outcome of one or more of the following findings: architectural abnormalities (villous atrophy or crypt hyperplasia), increased IEL or chronic inflammation. In this analysis, individuals who met one or more of these aforementioned criteria were collectively compared, via χ^2 testing, to those who met none of these criteria.

All p values reported are two-sided. We used SAS V.9.3 (Cary, North Carolina, USA) for statistical calculations.

RESULTS

Among the 20 olmesartan users, the mean age was 59.5 years and 70% were women (table 1).

Among 20 non-olmesartan ARB users, the mean age was 58.5 years and 55% were women. The indication for OGD was abdominal pain in all cases and controls. When we compared duodenal biopsies of olmesartan users with controls, we identified no single histopathological finding that was significantly more frequent in either group (table 2).

However, there were variables and a composite outcome which showed trends towards significance. Of note, 10 of 20 olmesartan-exposed patients (50%) had one or more of the following sprue-like features: architectural distortion (villous atrophy and/or crypt hyperplasia), generalised increase in IEL and chronic inflammation (figure 1A–C). This compares with 4 of 20 control patients (10%, p=0.10). Regarding individual findings, olmesartan users had more positive findings than control patients for each variable investigated (other than increased subepithelial collagen which was not seen in any case or control), though none achieved statistical significance. Specifically, 25% of olmesartan users had foci of villous atrophy compared with 6% of control patients (p=0.33). The mean maximum IEL count was 13.7 in the olmesartan group compared with 10.6 for controls (p=0.09). Certain other features also were more common in olmesartan users than in control patients, but they too failed to reach statistical significance. The most notable of these was increased crypt apoptosis, which was seen in 25% of olmesartan users compared with 10% of controls (figure 1D).

We also compared duodenal biopsies from individuals taking ARBs other than olmesartan with patients taking no ARB. There were no statistically significant differences and no trends that suggested a similar effect (table 2).

DISCUSSION

Olmesartan is a widely prescribed ARB used in the management of hypertension. Rarely, patients taking this drug develop a life-threatening diarrheal illness with duodenal biopsies that reveal a severe enteropathy often with increased collagen deposition.[1] A study performed at our institution showed that over 10 years, 72 patients had been referred with a diagnosis of seronegative villous atrophy (negative coeliac disease serologies). The most common diagnosis in this group was seronegative coeliac disease (20 patients who had coeliac disease associated human leucocyte antigen haplotypes and responded to a gluten-free diet). The second most common diagnosis (n=19) was medication-related enteropathy. Sixteen patients had olmesartan exposure and had similar clinical and histological findings as described in the Mayo Clinic series. Eleven of the 16 olmesartan-exposed patients had increased subepithelial collagen.[2] Of considerable relevance to our study is a case reported by Talbot. The patient described was taking olmesartan, but did not have diarrhoea (presented with constipation). The patient had multiple endoscopies with biopsy. The first duodenal biopsy showed normal duodenal architecture

Table 1 Patient characteristics

	Olmesartan analysis		Other ARB analysis	
	Olmesartan users (n=20)	Matched controls (n=20)	Other ARB users (n=20) Losartan: 11 Valsartan: 3 Telmisartan: 3 Irbesartan: 2 Candesartan: 1	Matched controls (n=20)
Age (median, range)	59.5 (48–76)	59.5 (48–76)	58.5 (35–84)	58.5 (35–84)
Gender				
Male	6 (30)	6 (30)	9 (45)	9 (45)
Female	14 (70)	14 (70)	11 (55)	11 (55)

ARB, angiotensin receptor blocker.

Table 2 Histological features of olmesartan and other ARB users compared with controls

	Olmesartan analysis			Other ARB analysis		
	Olmesartan users (n=20) (%)	Matched controls (n=20) (%)	p Value	Other ARB users (n=20) (%)	Matched controls (n=20) (%)	p Value
Villous atrophy	4/16 (25)*	1/16 (6)	0.33	1/14 (7)*	2/19 (11)	1.0
Crypt hyperplasia	4/16 (25)*	2/17 (12)	0.40	3/14 (21)*	4/18 (22)	1.0
Mean maximum IEL count	13.7	10.6	0.09	13.0	18.5	0.35
Generalised IEL increase	4/20 (20)	2/20 (10)	0.67	2/20 (10)	6/20 (30)	0.24
Chronic inflammation	5/20 (25)	2/20 (10)	0.40	7/20 (35)	6/20 (30)	1.0
Eosinophilia	2/20 (10)	0/20 (0)	0.49	3/20 (15)	2/20 (10)	1.0
Neutrophilia	8/20 (40)	6/20 (30)	0.74	4/20 (20)	7/20 (35)	0.48
Increased crypt apoptosis	5/20(25)	2/20 (10)	0.40	6/20 (30)	8/20 (40)	0.74
One or more sprue-like features (architectural abnormalities, generalised increased IEL, chronic inflammation)	10/20 (50)	4/20 (20)	0.10	9/20 (45)	12/20 (60)	0.34

*Villous atrophy and crypt hyperplasia was not evaluated in 4 olmesartan cases and in 6 ARB cases due to poor orientation.
ARB, angiotensin receptor blocker; IEL, intraepithelial lymphocyte.

but had increased lamina propria lymphoplasmacytic inflammation and IEL. A subsequent biopsy was similar, although showed 'mild villous blunting.' Based on the reports previously described, this patient was taken off olmesartan despite the lack of significant symptoms.[16] It is intriguing to consider whether this patient would have developed the 'full-blown' clinical and histological syndrome if he had continued to take this agent. Also of particular relevance to this study is a case, which showed similar clinical and pathological characteristics as were described in the Mayo series of olmesartan patients in a patient taking another ARB, valsartan.[17]

To determine whether olmesartan usage was associated with intestinal damage, short of the severe sprue-like enteropathy, we identified patients with abdominal pain who were taking olmesartan or other ARBs and had a duodenal biopsy. We demonstrated a trend towards sprue-like enteropathic changes in individuals taking olmesartan compared with controls. The trend towards increased crypt apoptosis is interesting mechanistically, as certain other drugs known to cause intestinal damage often demonstrate this finding (e.g. mycophenolate mofetil).[18] These changes appear to be specific for olmesartan as there were none identified in those taking other ARBs.

This is the first study to our knowledge that investigates whether exposure to olmesartan or other ARBs is associated with histopathological abnormalities among outpatients

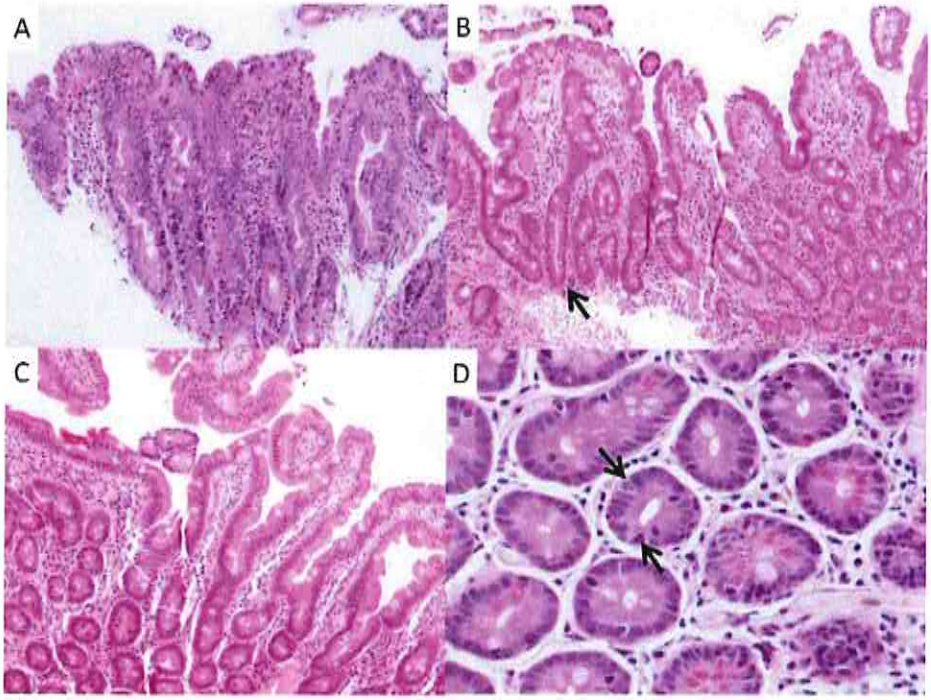

Figure 1 Highlighted findings in olmesartan users. (A) Representative photomicrograph of a small bowel biopsy from an individual showing one of several foci of villous atrophy, this particular case shows total villous atrophy but lacks intraepithelial lymphocytosis (H&E 200×). (B) A case with milder findings, including mild villous atrophy and focally pronounced crypt hyperplasia (arrow; H&E 100×). (C) This case had normal architecture, but a mild, generalised increase in intraepithelial lymphocytes (H&E 200×). (D) The case depicted in panel C also showed increased crypt apoptosis, including a crypt with 3–4 apoptotic bodies (arrows; H&E 600×).

undergoing duodenal biopsy. Our study has several limitations including its retrospective design, single centre setting and lack of information regarding duration of ARB use. We did not systematically exclude patients with known microscopic colitis; however, a post-hoc review showed that only 1 of 80 patients had microscopic colitis in our records (olmesartan user with no histopathological findings in our study). A larger sample size may have been useful, as it is possible that olmesartan causes a true increase in duodenal histopathological abnormalities but that our study was underpowered to detect this effect. Finally, we do not know whether any of the patients has subsequently discontinued olmesartan, and if so, if their abdominal pain has resolved.

This study raises the possibility that there may be a spectrum of injury associated with olmesartan use, apart from the severe syndrome that causes life-threatening diarrhoea. Further studies are needed to determine whether olmesartan use is associated with abdominal pain or other gastrointestinal symptoms and signs, as opposed to the well-characterised diarrhoea with sprue-like enteropathy. Future studies should follow-up the patients in this study to determine whether any of the olmesartan-exposed patients develop the severe enteropathic phenotype and if any of the histopathological variables we investigated are predictive thereof.

Take home messages

- This study raises the possibility that there is a spectrum of duodenal injury associated with olmesartan use.
- Angiotensin receptor blockers other than olmesartan are not associated with any histopathological findings in duodenal biopsies of patients with abdominal pain.
- Further studies are needed to determine whether olmesartan use is associated with abdominal pain and if the patients with the histopathological findings described here are at risk for developing the recently described severe sprue-like enteropathy.

Contributors SML: concept development, data collection, drafter of manuscript and guarantor of data. EDB: data collection and manuscript review. CA-G: concept development and manuscript review. GB: concept development and manuscript review. PG: concept development and manuscript review. BL: concept development, data analysis (statistics) and manuscript review.

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REFERENCES

1 Rubio-Tapia A, Herman ML, Ludvigsson JF, *et al*. Severe spruelike enteropathy associated with olmesartan. *Mayo Clin Proc* 2012;87:732–8.
2 DeGaetani M, Tennyson CA, Lebwohl B, *et al*. Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma. *Am J Gastroenterol*. 2013;108:647–53.
3 Abdelghany M, Gonzalez L 3rd, Slater J, *et al*. Olmesartan associated sprue-like enteropathy and colon perforation. *Case Rep Gastrointest Med* 2014;2014:494098.
4 de Fonseka A, Tuskey A, Moskaluk C. A case of olmesartan induced enteropathy. *Inflamm Bowel Dis* 2012;18(Suppl 17).
5 Dreifuss SE, Tomizawa Y, Farber NJ, *et al*. Spruelike enteropathy associated with olmesartan: an unusual case of severe diarrhea. *Case Rep Gastrointest Med* 2013;2013:618071.
6 Fiorucci G, Puxeddu E, Colella R, *et al*. Severe spruelike enteropathy due to olmesartan. *Rev Esp Enferm Dig* 2014;106:142–4.
7 Gaur V, Albeldawi M, Weber L. Chronic diarrhea and weight loss. *Gastroenterology* 2014;146:347,591.
8 Ianiro G, Bibbo S, Montalto M, *et al*. Systematic review: sprue-like enteropathy associated with olmesartan. *Aliment Pharmacol Ther*. 2014;40:16–23.
9 Khan AS, Peter S, Wilcox CM. Olmesartan-induced enteropathy resembling celiac disease. *Endoscopy* 2014;46(Suppl 1 UCTN):E97–8.
10 Nielsen JA, Steephen A, Lewin M. Angiotensin-II inhibitor (olmesartan)-induced collagenous sprue with resolution following discontinuation of drug. *World J Gastroenterol* 2013;19:6928–30.
11 Stanich PP, Yearsley M, Meyer MM. Olmesartan-associated sprue-like enteropathy. *J Clin Gastroenterol*. 2013;47:894–5.
12 Theophile H, David XR, Miremont-Salame G, *et al*. Five cases of sprue-like enteropathy in patients treated by olmesartan. *Dig Liver Dis* 2014;46:465–9.
13 Greywoode R, Braunstein ED, Arguelles-Grande C, *et al*. Olmesartan, other antihypertensives, and chronic diarrhea among patients undergoing endoscopic procedures: a case-control study. *Mayo Clin Proc*. 2014;89:1239–43.
14 Shmidt E, Smyrk TC, Boswell CL, *et al*. Increasing duodenal intraepithelial lymphocytosis found at upper endoscopy: time trends and associations. *Gastrointest Endosc*. 2014;80:105–11.
15 Kakar S, Nehra V, Murray JA, *et al*. Significance of intraepithelial lymphocytosis in small bowel biopsy samples with normal mucosal architecture. *Am J Gastroenterol*. 2003;98:2027–33.
16 Talbot GH. Small bowel histopathologic findings suggestive of celiac disease in an asymptomatic patient receiving olmesartan. *Mayo Clin Proc*. 2012;87:1231–2; author reply 2.
17 Herman M, Rubio-Tapia A, Marietta E, *et al*. Severe Enteropathy in a Patient on Valsartan. *Am J Gastroenterol* 2013;108(Suppl1):S302.
18 Parfitt JR, Jayakumar S, Driman DK. Mycophenolate mofetil-related gastrointestinal mucosal injury: variable injury patterns, including graft-versus-host disease-like changes. *Am J Surg Pathol*. 2008;32:1367–72.

EXHIBIT 3

AP&T Alimentary Pharmacology and Therapeutics

We thank Dr Wu for providing a balanced review[1] of our article[2] concerning the overlap of certain functional gastrointestinal disorders (FGIDs) with atopic conditions. The key issues mentioned were also raised as limitations by ourselves in the article. The Rome criteria could not be employed to define the FGIDs; we applied the definitions used by general practitioners who treat the majority of FGID patients[3] and who do not commonly use formal criteria such as Rome.[4, 5] It is also true that we cannot be certain that all patients flagged as functional in our sample do not have organic diseases that might explain their symptoms. There are however data to suggest that organic disease is relatively rare in individuals who are diagnosed with functional disease.[6, 7] It therefore seems likely that the influence of a relatively small proportion of possible organic disease patients will be diluted by a very large number of truly functional disease patients. Furthermore, our data are consistent with evidence which links a common pattern of immune activation in the gut and lung.[8]

While our data provide evidence of overlap between FGIDs and atopy, there remains a clear need to replicate this study in well-characterised patients using standardised measurement methodologies. It is arguably even more important to further study the mechanisms that drive the overlap and to understand whether the overlap is driven by the gut, atopy or an entirely different pathway.

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The authors' declarations of personal and financial interests are unchanged from those in the original article.[2]

REFERENCES

1. Wu JC-Y. Editorial: the overlap of atopy and functional gastrointestinal disorders in primary care. *Aliment Pharmacol Ther* 2014; **40**: 1244.
2. Jones MP, Walker MM, Ford AC, Talley NJ. The overlap of atopy and functional gastrointestinal disorders among 23 471 patients in primary care. *Aliment Pharmacol Ther* 2014; **40**: 382–91.
3. Guthrie E, Thompson D. Abdominal pain and functional gastrointestinal disorders. *BMJ* 2002; **325**: 701–3.
4. Olafsdottir LB, Gudjonsson H, Jonsdottir HH, *et al.* Irritable bowel syndrome: physicians' awareness and patients' experience. *World J Gastroenterol* 2012; **18**: 3715–20.
5. Vandvik PO, Aabakken L, Farup PG. Diagnosing irritable bowel syndrome: poor agreement between general practitioners and the Rome II criteria. *Scand J Gastroenterol* 2004; **39**: 448–53.
6. Cash BD, Schoenfeld P, Chey WD. The utility of diagnostic tests in irritable bowel syndrome patients: a systematic review. *Am J Gastroenterol* 2002; **97**: 2812–9.
7. Thompson WG, Heaton KW, Smyth GT, Smyth C. Irritable bowel syndrome in general practice: prevalence, characteristics, and referral. *Gut* 2000; **46**: 78–82.
8. Walker MM, Powell N, Talley NJ. Atopy and the gastrointestinal tract–a review of a common association in unexplained gastrointestinal disease. *Expert Rev Gastroenterol Hepatol* 2014; **8**: 289–99.

Editorial: sprue-like enteropathy due to olmesartan and other angiotensin receptor blockers – the plot thickens

B. Lebwohl*,† & J. F. Ludvigsson†,‡

*Celiac Disease Center, Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA.
†Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.
‡Department of Pediatrics, Örebro University Hospital, Örebro, Sweden.
E-mail: bl114@columbia.edu

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After investigators at the Mayo Clinic reported on 22 patients taking olmesartan who developed sprue-like enteropathy,[1] additional case reports and series were published to confirm this association.[2] Marthey *et al.* now report on the results of a national case-finding initiative in which gastroenterologists throughout France were asked to submit the clinical details of patients with sprue-like enteropathy due to olmesartan (or other angiotensin receptor blockers).[3] They identified 36 patients with olmesartan-associated enteropathy, and one with a similar presentation in the context of irbesartan use. Strengths of this study include its multicenter setting and the inclusion of patients with similarly severe clinical findings but more subtle histological abnormalities, such as the presence of intraepithelial lymphocytosis with normal villi.

How common is this adverse medication effect? The answer depends on the population under consideration. If the denominator consists of all individuals prescribed olmesartan or other angiotensin receptor blockers, sprue-like enteropathy appears to be very rare. Indeed, in an analysis of a randomised controlled trial involving

2232 patients prescribed olmesartan and monitored for adverse effects, there was no difference between those given olmesartan and those given placebo with regard to the development of diarrhoea or other gastrointestinal symptoms.[4] While that lack of association may be due to the relatively short observation time of a randomised trial, the low number of patients identified by Marthey *et al.* in the context of its national reach likewise underscores the notion that this outcome is rare. However, when one considers a more specialised population, such as those referred to a coeliac disease centre for seronegative villous atrophy[5] or those with collagenous sprue,[6] olmesartan appears to be a prominent, even common, cause of these uncommon conditions.

It is less certain whether this entity represents the 'tip of the iceberg,' – that is, whether more subtle symptoms and/or abnormal histology may be induced by olmesartan, and whether angiotensin receptor blockers apart from olmesartan may induce such outcomes. A prior case report of valsartan-induced enteropathy,[7] and the inclusion of irbesartan in the study by Marthey *et al.*, suggest that other angiotensin receptor blockers may contribute. Our understanding of this entity is evolving (see Table 1); future studies should aim to characterise the full spectrum of gastrointestinal pathology associated with olmesartan, and possibly other angiotensin receptor blockers.

Table 1 | Features of sprue-like enteropathy induced by olmesartan and other angiotensin receptor blockers

Characteristic	Study type	Reference
Resolves with drug discontinuation	Case series	1
Common cause of seronegative villous atrophy and collagenous sprue	Cases series	5,6
Not a common cause of diarrhoea	RCT	4
	Case–control study	8
	National case-finding study	3
May cause more subtle histological abnormalities	Case report	9
	National case-finding study	3
May be caused by non-olmesartan ARBs	Case report	7
	National case-finding study	3

ARB, angiotensin receptor blocker; RCT, randomised controlled trial.

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REFERENCES

1. Rubio-Tapia A, Herman ML, Ludvigsson JF, *et al.* Severe spruelike enteropathy associated with olmesartan. *Mayo Clin Proc* 2012; **87**: 732–8.
2. Ianiro G, Bibbo S, Montalto M, Ricci R, Gasbarrini A, Cammarota G. Systematic review: sprue-like enteropathy associated with olmesartan. *Aliment Pharmacol Ther* 2014; **40**: 16–23.
3. Marthey L, Cadiot G, Seksik P, *et al.* Olmesartan-associated enteropathy: results of a national survey. *Aliment Pharmacol Ther* 2014.
4. Menne J, Haller H. Olmesartan and intestinal adverse effects in the ROADMAP study. *Mayo Clin Proc* 2012; **87**: 1230–1.
5. DeGaetani M, Tennyson CA, Lebwohl B, *et al.* Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma. *Am J Gastroenterol* 2013; **108**: 647–53.
6. Rubio-Tapia A, Talley NJ, Gurudu SR, Wu TT, Murray JA. Gluten-free diet and steroid treatment are effective therapy for most patients with collagenous sprue. *Clin Gastroenterol Hepatol* 2010; **8**: 344–9.
7. Herman M, Rubio-Tapia A, Marietta E, Wu TT, Murray J. Severe enteropathy in a patient on valsartan. *Am J Gastroenterol* 2013; **108**: S302.
8. Greywoode R, Braunstein ED, Arguelles-Grande C, Green PH, Lebwohl B. Olmesartan, other antihypertensives, and chronic diarrhea among patients undergoing endoscopic procedures: a case-control study. *Mayo Clin Proc* 2014; **89**: 1239–43.
9. Talbot GH. Small bowel histopathologic findings suggestive of celiac disease in an asymptomatic patient receiving olmesartan. *Mayo Clinic Proc* 2012; **87**: 1231–2.

EXHIBIT 4

ORIGINAL ARTICLE

MAYO CLINIC

Blockers of Angiotensin Other Than Olmesartan in Patients With Villous Atrophy: A Nationwide Case-Control Study

Karl Mårild, MD, PhD; Benjamin Lebwohl, MD; Peter H.R. Green, MD; Joseph A. Murray, MD; and Jonas F. Ludvigsson, MD, PhD

Abstract

Objective: To examine the association between the previous use of nonolmesartan angiotensin receptor blockers (ARBs) or any angiotensin-converting enzyme inhibitor (ACEI) and subsequent villous atrophy (VA) in patients with small-intestinal VA as compared with general population–matched controls.

Patients and Methods: A case-control study was used to link nationwide histopathology data on 2933 individuals with VA (Marsh grade 3) to the Swedish Prescribed Drug Register to examine the association between the use of ACEIs as well as the specific use of ARBs other than olmesartan and subsequent VA. Olmesartan is not available in Sweden, so this exposure was not examined. All individuals with VA had biopsies performed between July 1, 2005, and January 29, 2008, and matched on age, sex, calendar period of birth, and county of residence to 14,571 controls from the general population.

Results: Use of nonolmesartan ARBs was not associated with VA (odds ratio, 0.84; 95% CI, 0.64-1.09; P=.19). Neither was VA associated with a previous medication of any ACEI (odds ratio, 1.08; 95% CI, 0.90-1.30; P=.41). Restricting the analysis to individuals with repeated prescriptions for ACEIs or ARBs revealed only marginally changed risk estimates for VA.

Conclusion: The lack of association between the use of ACEIs and nonolmesartan ARBs and subsequent VA suggests that these medications are not a major risk factor for the development of VA in the general population.

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From the Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway (K.M.); Department of Medical Epidemiology & Biostatistics, Karolinska Institutet, Stockholm, Sweden (K.M., J.F.L.); Celiac Disease Center, Department of Medicine, Columbia University Medical Center, Columbia University, New York, NY (B.L., P.H.R.G.); Division of Gastroenterology and Hepatology, Departments of Medicine and Immunology, Mayo Clinic, Rochester, MN (J.A.M.); and Department of Pediatrics, Örebro University Hospital, Örebro, Sweden (J.F.L.).

A duodenal biopsy showing villous atrophy (VA) has long been considered a diagnostic hallmark of celiac disease (also known as celiac sprue).[1] In celiac disease, dietary gluten causes small-intestinal VA and inflammation. Celiac disease is prevalent in 1% to 2% of the Western population.[1,2] Although celiac disease is by some margin the most common cause of VA, several additional causes of VA exist, for example, tropical sprue, infective gastroenteritis, and immunodeficiency states.[3]

In 2012, Rubio-Tapia et al[4] first described 22 patients taking olmesartan medoxomil, an angiotensin receptor blocker (ARB) used for the treatment of hypertension, who developed spruelike enteropathy. These patients, suffering from chronic diarrhea and weight loss accompanied with small-intestinal VA or inflammation, showed a marked clinical improvement after discontinuing olmesartan. Although these patients' intestinal histology resembled that of celiac disease, none of these patients had characteristics entirely consistent with celiac disease, that is, positive celiac disease serology and/or a symptomatic improvement on a gluten-free diet. Although questioned by some,[5,6] a number of case series[7,8] and 1 national case finding study[9] have since then reported additional cases of olmesartan-associated spruelike enteropathy. Some data have also suggested that other ARBs, besides olmesartan, may induce similar outcomes.[9] Drug-induced enteropathy is a challenging, often overlooked, differential diagnosis toward celiac disease. Despite this, there are few general population–based data on the previous use of angiotensin-converting enzyme inhibitors (ACEIs) and ARBs other than olmesartan before the development of VA.

The main objective of this study was to examine the association between the previous use of nonolmesartan ARBs as well as any ACEI and subsequent development of VA in patients

with small-intestinal VA as compared with general population–matched controls. To differentiate the use of these drugs in patients with VA, we also examined their usage in patients with VA as compared with individuals with milder small-intestinal histopathology: small-intestinal inflammation without VA or normal small-intestinal mucosa but positive celiac disease serology.[1]

PATIENTS AND METHODS

In this case-control study, we linked nationwide histopathology data on individuals undergoing small-intestinal biopsy to the Swedish Prescribed Drug Register to examine the association between the use of nonolmesartan ARBs or any ACEI and the subsequent development of VA.

Study Population

Between 2006 and 2008, we searched the computerized register of Sweden's 28 pathology departments to identify individuals with small-intestinal VA (Marsh grade 3).[10,11] The biopsies were performed between July 1969 and January 2008.[12] A detailed account of the data collection process has been described elsewhere.[10,13] In an earlier validation study on a randomly selected sample of patients in our cohort, 95% (108 of 114) of the patients with VA had later received a clinical diagnosis of celiac disease.[10]

In the present study, we used the same data set described in our previous study of mortality identifying 29,096 patients with VA.[14] The government agency Statistics Sweden then matched each individual with VA with up to 5 controls from the general population for age, sex, calendar period of birth, and county of residence. The number of controls was decided after consultations with the government agency Statistics Sweden. After the exclusion of individuals with data irregularities (see our previous report[14]), we identified 144,522 controls.

Patients with VA and their matched controls were then linked to the Swedish Prescribed Drug Register (established on July 1, 2005).[15] Through this linkage, we identified 2933 patients with VA who had biopsies performed between July 1, 2005 (the start of the Prescribed Drug Register), and January 29, 2008 (the end of the study period), and 14,571 matched controls.

Using Swedish computerized pathology data, we identified a secondary control group of individuals with small-intestinal inflammation (Marsh grades 1-2) but without VA and individuals with normal small-intestinal mucosa (Marsh grade 0) but positive celiac disease serology.[13] Data on individuals with normal mucosa and positive celiac disease serology were regional and obtained from the ascertainment areas of 8 Swedish university hospitals covering approximately half of the Swedish population.[13] *Positive celiac disease serology* was defined as a positive IgA or IgG antigliadin antibody, endomysial antibody, or tissue transglutaminase test less than 180 days before or no later than 30 days after a normal biopsy result (and with no previous or subsequent biopsy showing VA or inflammation).[13] In total, this secondary control group included 2738 individuals (2118 individuals with inflammation and 620 individuals with normal mucosa but positive celiac disease serology).

Use of ARBs and ACEIs

The Swedish Prescribed Drug Register contains prospectively recorded individual data on more than 99% of all dispensed prescribed drugs in Sweden.[15]

We collected data on the use of any ACEI (Anatomical Therapeutic Chemical [ATC] code, C09) as well as the specific use of ARBs other than olmesartan (ATC codes, C09C and C09D) from July 1, 2005 (launch of the Prescribed Drug Register), through January 29, 2008 (end of the study period), and up to the date of the biopsy (and the corresponding date in matched controls). Olmesartan is not available in Sweden, so this exposure was not studied in this population-based investigation.

Statistical Analyses

We used conditional logistic regression to estimate odds ratios (ORs) and 95% CIs. Each stratum (1 individual undergoing biopsy and up to 5 matched controls) was analyzed separately before a summary OR was calculated.[16] This statistical approach therefore eliminates the effect of sex, age, county, and calendar year on our ORs.

In analyses on the specific use of nonolmesartan ARBs and subsequent VA, other types of ACEIs were not considered. For the usage of both ARBs and any ACEI, we performed stratified analyses by sex and by age at the time of biopsy showing VA (0-19, 20-39, 40-59, and ≥60 years). In this study, we choose to also include children because national prescription

TABLE 1. Descriptive Characteristics of Individuals With Small-Intestinal Villous Atrophy[a]

Characteristic	Value
Total no. of patients	2933
Sex, n (%)	
Women	1796 (61.2)
Men	1137 (38.8)
Median age at study entry (y) (range)	28 (0-94)
Age (y), n (%)	
0-19	1218 (41.5)
20-39	566 (19.3)
40-59	583 (19.9)
60+	566 (19.3)
Year, n (%)	
2005[b]	819 (27.9)
2006	1828 (62.3)
2007[c]	274 (9.3)
2008[d]	12 (0.4)

[a]Reference individuals have not been included in the table because their age, sex, and entry year distributions were identical to those of individuals with villous atrophy (due to matching).

[b]Beginning of study period: July 1, 2005.

[c]Most of the pathology departments delivered data on individuals with small-intestinal pathology undergoing biopsy up to the beginning of year 2007. The remaining pathology departments reported histopathology data up to the end of 2007 or very early 2008. For this reason, our data included fewer individuals with villous atrophy who had biopsies performed in 2007 than in 2006.

[d]End of study period: January 29, 2008.

data indicate that more than 1000 Swedish children per year are treated with an ACEI.[17] To evaluate potential causality, we estimated the dose- and time-dependent association between ARB/ACEI medication and VA in 2 separate analyses: (1) when individuals had received at least 2 prescriptions of any ARB/ACEI and (2) when an ARB/ACEI had been prescribed at least 1 year (>365 days) before biopsy. Education level has been associated with overall drug utilization[18] and health care utilization (and ascertainment of small-intestinal VA).[19] In a subanalysis, we therefore adjusted for education using 7 predefined education categories determined by Statistics Sweden.

To differentiate the use of ARBs/ACEIs in patients with VA, we also examined their usage in individuals with small-intestinal inflammation without VA (Marsh grades 1-2) and individuals with normal small-intestinal mucosa (Marsh grade 0) but positive celiac disease serology. In this subanalysis, we used logistic regression adjusted for age at the time of biopsy (showing VA, inflammation, or normal mucosa), sex, and calendar year of study entry to estimate ORs and 95% CIs.

Post Hoc Analyses

Although most studies implicating drug-induced spruelike enteropathy implicate olmesartan, 2 studies have reported cases of VA associated with nonolmesartan ARBs, irbesartan and valsartan, respectively.[9,20] We, therefore, collected data on the specific use of irbesartan (ATC code, C09CA04) and valsartan (ATC code, C09CA03).

In a post hoc analysis, we specifically examined the association between the previous use of ARBs/ACEIs among 2118 individuals with small-intestinal inflammation without VA (Marsh grades 1-2) as compared with matched controls from the general population (n=10,442) (see matching procedure described above for patients with VA).

We have previously shown that patients with celiac disease with small-intestinal VA have a more favorable cardiac risk profile, including decreased risk of hypertension, as compared with the general population.[21] Therefore, to examine the susceptibility to confounding by indication, we contrasted the use of ARBs/ACEIs by examining the association between VA and previous antihypertensive therapy with calcium channel blockers. Data on the use of any calcium channel blocker (ATC code, C08) were collected from the Prescribed Drug Register between July 1, 2005, and January 29, 2008, and up to the date of biopsy showing VA (and the corresponding date in matched controls).

For analyses on the previous use of ARBs/ACEIs in individuals with VA, we examined for interactions between sex and exposure via the inclusion of multiplicative interaction terms in an unconditional logistic regression model adjusted for age, sex, and calendar year.

Statistical significance was defined as 95% CIs for risk estimates not including 1.0 and P values of <.05. SPSS (version 22.0) was used for all statistical analyses.

Ethics

This study was conducted in accordance with national and institutional standards and was approved by the Regional Ethical Vetting Board in Stockholm.

TABLE 2. Odds Ratios for Previous Use of ARBs in Individuals With Villous Atrophy as Compared With General Population–Matched Controls[a,b]

Charactenstic	Villous atrophy (%)	Controls (%)	Odds ratio	95% CI	P
ARBs[c]	66 of 2933 (2.3)	387 of 14,571 (2.7)	0.84	0.64-1.09	.19
Sex					
Male	41 of 1137 (3.6)	187 of 5645 (3.3)	1.09	0.77-1.55	.62
Female	25 of 1796 (1.4)	200 of 8926 (2.2)	0.61	0.40-0.92	.02
Repeated prescriptions of ARBs	64 of 2931 (2.2)	378 of 14,562 (2.6)	0.83	0.63-1.09	.18
Use of ARBs >1 y before biopsy	22 of 2889 (0.8)	119 of 14,303 (0.8)	0.93	0.59-1.49	.78

[a]ARB = angiotensin receptor blocker.
[b]Odds ratios estimated through conditional logistic regression. Through this statistical approach all analyses were carried out stratumwise and thereby conditioned on age at the time of biopsy (and corresponding date in controls), calendar period, sex, and county of residence.
[c]Use of ARBs (Anatomical Therapeutic Chemical code, C09C) between July 1, 2005, and January 29, 2008.

RESULTS

Of the 2933 individuals with VA, some 60% were women. The median age at biopsy was 28 years (1715/2933 [58.5%] of those with VA had biopsies performed in adulthood) (Table 1).

Use of ARBs

A total of 66 individuals with VA (2.3%) and 387 controls (2.7%) had an earlier record of medication with a nonolmesartan ARB, equivalent to an OR of 0.84 for subsequent VA (95% CI, 0.64-1.09). None of the children with VA had a previous treatment with an ARB. Among adults with VA, ORs did not differ appreciably according to age at the time of biopsy (Supplemental Table 1, available online at http://www.mayoclinicproceedings.org). Adjustment for education level revealed an unchanged OR (adjusted OR, 0.84; 95% CI, 0.64-1.11; P=.22). As compared with sex-matched controls, we found a significantly decreased risk estimate for VA in women with previous treatment with an ARB (OR, 0.61; 95% CI, 0.40-0.92) that was not found in men (OR, 1.09; 95% CI, 0.77-1.55). The P value for interaction (sex×ARB) in an unconditional logistic regression model was .04. We found no association between VA and repeated prescriptions of ARBs or treatment initiated at least 1 year (>365 days) before biopsy (Table 2).

ORs for the previous use of ARBs did not differ appreciably according to calendar year at the time of biopsy (Supplemental Table 2, available online at http://www.mayoclinicproceedings.org).

USE OF ANY ACEI

Of the 2933 individuals with VA, 165 (5.6%) had received at least 1 prescription of any ACEI before biopsy showing VA as compared with 762 of 14,571 (5.2%) among the general population–based controls, corresponding to an OR of 1.08 (95% CI, 0.90-1.30) for subsequent development of VA (Table 3). Restricting our analysis to individuals with VA who had biopsies performed in adulthood, we found largely unchanged risk estimates (OR, 1.08; 95% CI, 0.89-1.30; P=.44). Adjustment for education level revealed largely unchanged OR (adjusted OR, 1.12; 95% CI, 0.93-1.35; P=.25). The association between the use of any ACEI and subsequent VA was similar in men and women (men: OR, 1.22, 95% CI, 0.95-1.56; women: OR, 0.94; 95% CI, 0.71-1.25), as compared with sex-matched controls. The P value for interaction (sex×ACEI) in an unconditional logistic regression model was .21.

We found no indication of a dose-response effect for individuals with repeated prescriptions of ACEIs (OR, 1.06; 95% CI, 0.88-1.28). As expected, treatment with ACEIs was very rare among children and was increasingly more common according to age at the time of biopsy. Among those aged 20 to 39 years at the time of biopsy, 6 individuals with VA (1.1%), as compared with 7 controls (0.2%), had previously been treated with any ACEI (OR, 3.82; 95% CI, 1.41-10.38). In none of the remaining age bands, nor in stratified analyses by calendar year at time of biopsy, did we find an association between the previous use of ACEIs and subsequent development of VA (Supplemental Table 3 and Supplemental Table 4, respectively, available online at http://www.mayoclinicproceedings.org).

TABLE 3. Odds Ratios for Previous Use of Any ACEI in Individuals With Villous Atrophy as Compared With General Population–Matched Controls[a,b]

Characteristic	Villous atrophy (%)	Controls (%)	Odds ratio	95% CI	P
Any ACEI[c]	165 of 2933 (5.6)	762 of 14,571 (5.2)	1.08	0.90-1.30	.41
Sex					
Male	99 of 1137 (8.7)	418 of 5645 (7.4)	1.22	0.95-1.56	.12
Female	66 of 1796 (3.7)	344 of 8926 (3.9)	0.94	0.71-1.25	.66
Repeated prescriptions of any ACEI	160 of 2928 (5.5)	751 of 14,560 (5.2)	1.06	0.88-1.28	.52
Use of ACEI >1 y before biopsy	47 of 2815 (1.7)	238 of 14,047 (1.7)	1.01	0.72-1.41	.98

[a]ACEI = angiotensin-converting enzyme inhibitor.

[b]Odds ratios estimated through conditional logistic regression. Through this statistical approach all analyses were carried out stratumwise and thereby conditioned on age at the time of biopsy (and corresponding date in controls), calendar period, sex, and county of residence.

[c]Any ACEI (Anatomical Therapeutic Chemical code, C09) used between July 1, 2005, and January 29, 2008.

Subanalyses

In a number of preplanned subanalyses, we also examined the use of ARBs/ACEIs in patients with VA as compared with individuals with small-intestinal inflammation without VA and individuals with normal small-intestinal mucosa but positive celiac disease serology. Overall, we identified 2738 individuals with these potentially prodromal stages of VA. In this secondary control group, 1732 (63%) were women and the median age at the time of biopsy was 41 years.

Using logistic regression analysis adjusting for sex, age, and calendar year of study entry, we found only marginally changed ORs for the previous use of any ACEI in individuals with VA as compared with individuals with mucosal inflammation or with normal biopsy result but positive celiac disease serology (adjusted OR, 1.08; 95% CI, 0.87-1.35) (Supplemental Table 5, available online at http://www.mayoclinicproceedings.org). Neither did we find a statistically significant association between VA and the repeated use of any ACEI medication as compared with individuals with mucosal inflammation or normal mucosa but positive celiac disease serology (adjusted OR, 1.07; 95% CI, 0.85-1.34).

Overall, the use of ARBs was not related with subsequent development of VA as compared with individuals with small-intestinal inflammation or normal mucosa but positive celiac disease serology (Supplemental Table 6, available online at http://www.mayoclinicproceedings.org).

Post Hoc Analyses

In a post hoc analysis, 7 individuals with VA (0.2%) and 37 controls (0.3%) had an earlier record of irbesartan (ATC code, C09CA04), equivalent to an OR of 0.93 for subsequent development of VA (95% CI, 0.42-2.09; P=.87). Looking specifically at the earlier use of valsartan (VA: 4 of 2933 [0.1%]; controls: 38 of 14,571 [0.3]) revealed a slightly lower OR for subsequent development of VA (OR, 0.52; 95% CI, 0.19-1.44; P=.21).

Of the 2118 individuals with small-intestinal inflammation without VA, 111 (5.2%) had an earlier record of medication with a nonolmesartan ARB, as compared with 341 of 10,442 (3.3%) controls from the general population (OR, 1.63; 95% CI, 1.31-2.03; P<.001). We largely found similarly increased ORs for subsequent small-intestinal inflammation without VA after repeated prescriptions of ARBs (OR, 1.62; 95% CI, 1.30-2.02; P<.001); however, we found no increased risk after ARB treatment initiated at least 1 year (>365 days) before biopsy (OR, 1.09; 95% CI, 0.73-1.64; P=.66). In individuals with intestinal inflammation without VA, OR for previous ACEI treatment was 1.57 (95% CI, 1.33-1.86; P<.001) (repeated use of ACEIs: OR, 1.57; 95% CI, 1.32-1.86; P<.001; ACEI treatment initiated at least 1 year before biopsy: OR, 1.17; 95% CI, 0.88-1.58; P=.28).

Finally, to contrast the use of ARBs/ACEIs, we examined the previous use of calcium channel blockers in individuals with VA (86 of 2933 [2.9%]) as compared with general population–based controls (502 of 14,571 [3.4%]) (OR, 0.83; 95% CI, 0.66-1.06; P=.13)

DISCUSSION

In this study, we examined the association between blockers of the angiotensin pathway and VA. Our study involved almost 3000

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individuals with VA, and overall we found no positive association between the previous use of ARBs or ACEIs and the subsequent development of VA in the general population; nor did we find a relationship between these drugs and VA when restricting our definition of exposure to multiple prescriptions. Neither did we find an association between the previous use of ARBs/ACEIs in individuals with VA and the subsequent development of VA as compared with individuals with milder small-intestinal histopathology.

Olmesartan is not used in Sweden, but a large number of individuals are treated with nonolmesartan ARBs and a positive finding here would have larger health implications than an effect restricted to olmesartan. Although the bulk of recent case reports and series implicating drug-induced spruelike enteropathy implicate olmesartan, a recent French study[9] included 1 case of nonolmesartan (irbesartan)-associated VA, and there are also case reports of valsartan- and telmisartan-associated VA.[20,22] Subtle histologic abnormalities short of VA have been reported with the use of olmesartan, but not with the use of other ARBs.[23] It therefore has been a pressing concern whether this recently described spruelike enteropathy is a class effect or is unique to (or more closely associated with) olmesartan. Our study, which includes 2933 patients with VA and 14,571 matched controls who were exposed to ACEIs and nonolmesartan ARBs, found no association between these drugs and VA.

Olmesartan appears to cause a spruelike enteropathy, but it has not been shown to trigger celiac disease per se. In a chart validation of a randomly selected sample of patients from our cohort, 95% of those with VA later received a clinical diagnosis of celiac disease.[10] However, it is likely that before the first report of this clinical entity in June 2012,[4] patients with this condition would be misdiagnosed with celiac disease. Indeed, the initial case series describing olmesartan-associated enteropathy arose from referral centers for celiac disease because many of these patients were initially thought to have nonresponsive or refractory celiac disease.[4,24] Therefore, we believe that a spruelike enteropathy would be detectable in an analysis of patients with VA who had biopsies performed before 2012. The fact that we found no association between the use of ARBs/ACEIs and VA suggests that spruelike enteropathy is not commonly triggered by these drugs.

Instead, the findings of our study are more consistent with the randomized clinical trial by Menne and Haller[6] who were unable to detect an increased risk of enteropathy in patients prescribed olmesartan. That study included a median follow-up of 3.2 years, and olmesartan-associated enteropathy can develop after even 10 years of drug exposure.[9] It is possible that nonolmesartan ARBs may trigger an enteropathy that we were unable to detect because of the relatively short drug exposure time in our study.

Our null findings in regard of subsequent development of VA can be interpreted in several ways. First, the available nonolmesartan drugs used in Sweden may not be associated with VA. The mechanism underlying olmesartan-induced enteropathy is unknown, but it has been hypothesized to be the result of a proapoptotic effect of angiotensin II on intestinal epithelial cells.[7] Speculatively, this apoptotic effect may hence be limited to olmesartan. Second, several articles have linked olmesartan to serology-negative VA.[24] Our data collection was based on mucosal abnormalities and not primarily serology, but an earlier validation of a subset of patients with VA from our cohort found that 88% had a positive celiac serology at the time of biopsy (defined here as tissue transglutaminase test/endomysial antibody but also positive antigliadin antibody because our cohort stretches back to 1969).[10] On interviewing 180 gastroenterologists and 68 pediatricians at the time of data collection (year 2008), 86% and 100%, respectively, reported that a positive serology was part of their diagnostic algorithm in at least 8 of 10 patients.[10] Hence the proportion of serology-negative individuals in our study is low, potentially adding to our null findings. Third, as noted above, if ARBs induce VA only after a long period of use, we may have missed a positive association. The Swedish Prescribed Drug Register that was used to ascertain ARB medication has been in use only since mid-2005 and hence we had a short follow-up of patients.

Because patients with celiac disease with small-intestinal VA may have a reduced risk of hypertension,[21,25] we carried out a sensitivity analysis revealing no statistically significant association (P=.13) between VA and previous treatment with calcium channel blockers. These results argue against confounding by indication as a sole cause of our null findings.

In post hoc analyses, we found positive associations between subsequent small-intestinal inflammation without VA and previous treatment with ARBs/ACEIs. However, these statistically significant increased risk estimates were confined to treatment initiated within 1 year before biopsy and one explanation for these findings could be that some individuals with multiple preexisting morbidity (including cardiovascular disease) undergo small-intestinal biopsy as part of a general investigation.

This study has some strengths and limitations. Among the strengths are the large numbers of patients with VA and that data on ARB use were collected from an independent source (the Swedish Prescribed Drug Registry). Although we cannot rule out that a small proportion of individuals with VA in this study were false-positive (an earlier blinded validation study found that Swedish pathologists correctly identify 90% of all VA cases),[10] a misclassification rate of 10% should not drive the risk estimate down to 1.08 (95% CI=0.90-1.30) and 0.84 (95% CI=0.64-1.09) for previous use of ACEIs and ARBs, respectively.

Although olmesartan has often been linked to clinically severe celiac like enteropathy,[4] we lacked individual-based information on symptom severity in our participants. However, when examining the patient charts of 118 random individuals with VA, some 79% had gastrointestinal symptoms. Hence, it is unlikely that our null findings are due to lack of classical symptoms[2] in our cohort. If nonolmesartan ARBs cause enteropathy as a very rare, long-term adverse effect, our study is unlikely to have the statistical power or follow-up time to detect this effect.

CONCLUSION

We found no increased risk of VA in Swedish individuals with a previous record of nonolmesartan ARB use or ACEI use. Future studies should elucidate the distinct features by which olmesartan, more so than other members of this drug class, induces VA.

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org.

Abbreviations and Acronyms: **ACEI** = angiotensin-converting enzyme inhibitor; **ARB** = angiotensin receptor blocker; **ATC** = Anatomical Therapeutic Chemical (pharmaceutical classification); **OR** = odds ratio; **VA** = villous atrophy

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Correspondence: Address to Karl Mårild, MD, PhD, Division of Epidemiology, Norwegian Institute of Public Health, PO Box 4404, Nydalen, 0403 Oslo, Norway (karlmarild@gmail.com).

REFERENCES

1. Ludvigsson JF, Leffler DA, Bai JC, et al. The Oslo definitions for coeliac disease and related terms. *Gut*. 2013;62(1):43-52.
2. Walker MM, Murray JA, Ronkainen J, et al. Detection of celiac disease and lymphocytic enteropathy by parallel serology and histopathology in a population-based study. *Gastroenterology*. 2010;139(1):112-119.
3. Dewar DH, Ciclitira PJ. Clinical features and diagnosis of celiac disease. *Gastroenterology*. 2005;128(4, Suppl 1):S19-S24.
4. Rubio-Tapia A, Herman ML, Ludvigsson JF, et al. Severe spruelike enteropathy associated with olmesartan. *Mayo Clin Proc*. 2012;87(8):732-738.
5. Greywoode R, Braunstein ED, Arguelles-Grande C, Green PH, Lebwohl B. Olmesartan, other antihypertensives, and chronic diarrhea among patients undergoing endoscopic procedures: a case-control study. *Mayo Clin Proc*. 2014;89(9):1239-1243.
6. Menne J, Haller H. Olmesartan and intestinal adverse effects in the ROADMAP study. *Mayo Clin Proc*. 2012;87(12):1230-1231; author reply 1232.
7. Ianiro G, Bibbò S, Montalto M, Ricci R, Gasbarrini A, Cammarota G. Systematic review: sprue-like enteropathy associated with olmesartan. *Aliment Pharmacol Ther*. 2014;40(1):16-23.
8. Bhat N, Anupama NK, Yelsangikar A, Vizhi K. Olmesartan-related sprue-like enteropathy. *Indian J Gastroenterol*. 2014;33(6):564-567.
9. Marthey L, Cadiot G, Seksik P, et al. Olmesartan-associated enteropathy: results of a national survey. *Aliment Pharmacol Ther*. 2014;40(9):1103-1109.
10. Ludvigsson JF, Brandt L, Montgomery SM, Granath F, Ekbom A. Validation study of villous atrophy and small intestinal inflammation in Swedish biopsy registers. *BMC Gastroenterol*. 2009;9:19.
11. Marsh MN. Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge. *Gut*. 1990;31(1):111-114.
12. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls

in healthcare and medical research. *Eur J Epidemiol.* 2009; 24(11):659-667.
13. Ludvigsson JF, Brandt L, Montgomery SM. Symptoms and signs in individuals with serology positive for celiac disease but normal mucosa. *BMC Gastroenterol.* 2009;9:57.
14. Ludvigsson JF, Montgomery SM, Ekbom A, Brandt L, Granath F. Small-intestinal histopathology and mortality risk in celiac disease. *JAMA.* 2009;302(11):1171-1178.
15. Wettermark B, Hammar N, Fored CM, et al. The new Swedish Prescribed Drug Register—opportunities for pharmacoepidemiological research and experience from the first six months. *Pharmacoepidemiol Drug Saf.* 2007;16(7):726-735.
16. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology.* 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.
17. Statistics Sweden. Statistical database. www.socialstyrelsen.se/statistik/statistikdatabas. Accessed December 2, 2014.
18. Weitoft GR, Rosén M, Ericsson O, Ljung R. Education and drug use in Sweden—a nationwide register-based study. *Pharmacoepidemiol Drug Saf.* 2008;17(10):1020-1028.
19. Westin M, Ahs A, Bränd Persson K, Westerling R. A large proportion of Swedish citizens refrain from seeking medical care—lack of confidence in the medical services a plausible explanation? *Health Policy.* 2004;68(3):333-344.
20. Herman ML, Rubio-Tapia A, Wu T, Murray JA. A case of severe sprue-like enteropathy associated with valsartan. *ACG Case Rep J.* 2015;2(2):92-94.
21. Emilsson L, Carlsson R, Holmqvist M, James S, Ludvigsson JF. The characterisation and risk factors of ischaemic heart disease in patients with coeliac disease. *Aliment Pharmacol Ther.* 2013; 37(9):905-914.
22. Cyrany J, Vasatko T, Machac J, Nova M, Szanyi J, Kopacova M. Letter: telmisartan-associated enteropathy—is there any class effect? *Aliment Pharmacol Ther.* 2014;40(5):569-570.
23. Lagana SM, Braunstein ED, Arguelles-Grande C, Bhagat G, Green PH, Lebwohl B. Sprue-like histology in patients with abdominal pain taking olmesartan compared with other angiotensin receptor blockers. *J Clin Pathol.* 2015;68(1):29-32.
24. DeGaetani M, Tennyson CA, Lebwohl B, et al. Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma. *Am J Gastroenterol.* 2013;108(5):647-653.
25. West J, Logan RF, Card TR, Smith C, Hubbard R. Risk of vascular disease in adults with diagnosed coeliac disease: a population-based study. *Aliment Pharmacol Ther.* 2004;20(1):73-79.

EXHIBIT 5

AP&T Alimentary Pharmacology and Therapeutics

Olmesartan-associated enteropathy: results of a national survey

L. Marthey*,†, G. Cadiot‡, P. Seksik§, P. Pouderoux¶, J. Lacroute**, F. Skinazi††, B. Mesnard‡‡, J. A. Chayvialle§§, G. Savoye¶¶, A. Druez***, D. Parlier†††, V. Abitbol‡‡‡, M. Gompel§§§, M. Eoche¶¶¶, E. Poncin****, R. Bobichon††††, P. Colardelle‡‡‡‡, P. Wils§§§§, H. Salloum¶¶¶¶, S. Peschard*****, F. Zerbib†††††, B. Méresse‡‡‡‡‡, N. Cerf-Bensussan‡‡‡‡‡, G. Malamut§§§§§ & F. Carbonnel*

*Kremlin Bicêtre University Hospital, Assistance Publique-Hopitaux de Paris (AP-HP), Paris-Sud University, Le Kremlin Bicêtre.
†Antoine Béclère University Hospital, AP-HP, Paris-Sud University, Clamart, France.
‡Robert Debré University Hospital, Reims, France.
§Saint-Antoine University Hospital, APHP, Sorbonne Universités - UPMC Univ Paris 6, Paris, France.
¶Caremeau University Hospital, Nimes, France.
**Sainte Barbe Clinic, Strasbourg, France.
††Delafontaine Hospital, Saint Denis, France.
‡‡Gustave Bron Hospital, Tourcoing, France.
§§Université Claude Bernard-Lyon 1, Lyon, France.
¶¶Charles Nicolle University Hospital, Rouen, France.
***Mont Godinne Hospital, Yvoir, Belgium.
†††Ambroise Paré University Hospital, Boulogne, France.
‡‡‡Cochin University Hospital, APHP, Paris, France.
§§§Kennedy Clinic, Montelimar, France.
¶¶¶Amiens University Hospital, Amiens, France.
****Dax-côte d'Argent Hospital, Dax, France.
††††Pays de Gier Hospital, Saint Chamond, France.
‡‡‡‡Versailles Hospital, Le Chesnay, France.
§§§§Oscar Lambret University Hospital, Lille, France.
¶¶¶¶Meaux Hospital, Meaux, France.
*****Meulan-les Mureaux Hospital, Meulan, France.
†††††Saint André University Hospital, Bordeaux Cedex, France.
‡‡‡‡‡INSERM, UMR 1163, Laboratory of Intestinal Immunity, Université Paris Descartes-Sorbonne Paris Centre, Paris, France.
§§§§§HEGP University Hospital, APHP, Paris, France.

Correspondence to:
Prof. F. Carbonnel, Department of Gastroenterology, Kremlin University Bicêtre Hospital, Assistance Publique-Hopitaux de Paris (AP-HP), Paris Sud University, 78 rue du Général Leclerc, 94275 Le Kremlin Bicêtre, France.
E-mail: franck.carbonnel@bct.aphp.fr

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SUMMARY

Background
Recently, a new enteropathy has been described: olmesartan-associated enteropathy. However, the association has been questioned: a phase 3 trial and a cohort study found no association between gastrointestinal events and olmesartan.

Aim
We collected French cases of sartan-associated enteropathy to describe further this entity, confirm or refute causality, and determine if the association exists with other sartans.

Methods
French gastroenterologists were invited to report cases of sartan-associated enteropathy and collect clinical, biological and histological data. Patients with diarrhoea and histological duodenal abnormalities were included.

Results
Thirty-six patients with olmesartan-associated enteropathy were reported, including 32 with villous atrophy and four without. There was only one patient with irbesartan-associated enteropathy. None of the patients died. Patients with villous atrophy had diarrhoea, vomiting, renal failure, hypokalaemia, body weight loss and hypoalbuminaemia. Thirty-one patients were hospitalised; four required intensive care. Anti-transglutaminase and anti-enterocyte antibodies were negative; anti-nuclear antibodies were positive (9/11). Endoscopic duodenal biopsies showed villous atrophy (32/32) and polyclonal intra-epithelial CD3+CD8+ lymphocytosis (11/11). Exactly, 14/15 patients responded to steroids and/or immunosuppressants, prescribed because of suspected autoimmune enteropathy. Ten olmesartan interruptions were followed by reintroductions before steroids or immunosuppressants. Interruptions were followed by remissions (9/10), but reintroductions were followed by relapses (9/9). Twenty-nine patients were in remission since olmesartan interruption, including 26 without immunosuppressants. Patients with normal villi had similar clinical characteristics, but mild histological abnormalities (intra-epithelial lymphocytosis and lamina propria lymphocytic infiltration).

Conclusions
Olmesartan causes a severe and immune-mediated enteropathy, with or without villous atrophy. Enteropathy associated with other sartans seems to be very rare.

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INTRODUCTION

Noncoeliac sprue encompasses several diseases[1]: tropical sprue,[2] refractory sprue,[3–9] low-grade T-cell lymphoma [10–12] and adult autoimmune enteropathy.[13, 14] More recently, Rubio-Tapia *et al.* have described 22 patients with severe, noncoeliac enteropathy who improved after discontinuation of olmesartan.[15] It has also been suggested that Olmesartan may account for a significant proportion of noncoeliac enteropathy, as reported in a series of 72 adult patients with villous atrophy and negative coeliac disease serology.[16] Sixteen of them were found to be treated with olmesartan, 15 of whom improved after olmesartan withdrawal. Several other cases have been reported.[17]

Olmesartan medoxomil, the prodrug of olmesartan, is an angiotensin II receptor blocker (ARB). It was approved in 2002 in the USA, and in 2003 in the European Union, for the treatment of hypertension. In the ROADMAP trial, a phase 3 trial performed in 4447 patients with diabetes mellitus, the rates of diarrhoea and abdominal discomfort were similar in the olmesartan and the placebo arm.[18, 19] More recently, a retrospective cohort analysis performed in 45 185 (116 721 patient-years) diabetic patients, compared olmesartan with other sartans and found no significant association between olmesartan and gastrointestinal disease-related hospitalisation.[20] Therefore, the association between enteropathy and olmesartan has been called into question.

Following the report by Rubio Tapia *et al.*,[15] we decided to perform a survey of enteropathy associated with olmesartan and other sartans in France. The aims of the present study were to describe further this new entity, to confirm or refute the causality of the association (by the study of olmesartan interruptions and reintroductions) and to determine if the enteropathy is associated with olmesartan only or with sartans in general.

PATIENTS AND METHODS

In July 2013, we sent an electronic alert to French gastroenterologists to inform them of the data reported by Rubio-Tapia *et al.* and invited them to report the cases of diarrhoea (either severe or not) associated with the use of sartans. We sent an email to Gastroenterologists working in University hospitals. In addition, a letter was sent to all Gastroenterologists, either in public hospitals or in private practice, using the electronic letter called Gastroscoop. This letter is published by the French National Society of Gastroenterology and sent to 2400 Gastroenterologists in France. We also contacted by email several Gastroenterology departments in French-speaking Belgium. Investigators were asked to report their observations to the pharmacovigilance unit to which their centre was affiliated and to participate in the cohort by filling out an anonymous, pre-specified electronic form. Clinical, biological and histological data were collected. Quantitative variables were expressed as median [range]. We included patients who had diarrhoea and abnormal histology on duodenal biopsies. We put the patients into two groups: patients with villous atrophy and those with normal villi and histological abnormalities such as intra-epithelial lymphocytosis or infiltration of lamina propria. The study was submitted to the ethical committee of Paris-Ile de France VII. This committee stated that there was no ethical issue related to this study.

RESULTS

Twenty-seven hospitals or medical centres reported 48 cases, including 47 with olmesartan and one with irbesartan. Data were available for 40 patients (completed electronic form), including 39 who received olmesartan and one who received irbesartan. One patient had normal duodenal biopsies and two patients did not have duodenal biopsies; these three patients were excluded from analysis.

Among the remaining 36 patients treated by olmesartan, 32 had villous atrophy and four had normal villi, but histological duodenal abnormalities.

Enteropathy with villous atrophy associated with olmesartan

Thirty-two cases (17 women) were reported. The patients were recruited in university hospitals (n = 23), general hospitals (n = 7) or private medical centres (n = 2). The median age was 70 years [46–91]. Seven patients had a past history of autoimmune or inflammatory disease (Table S1) and three patients had first-degree family history of autoimmune or inflammatory disease (two with coeliac disease, one with ulcerative colitis and one with rheumatoid arthritis). All patients received olmesartan for hypertension. Patients received six different formulations of olmesartan, including two in which olmesartan was combined with diuretics. The median dosage of olmesartan was 40 mg/day [10–60]. Sixteen patients received drugs other than olmesartan before the first symptoms of enteropathy. Concomitant medications differed between patients (Table S2). Therefore, there were no specific associations between olmesartan, other drugs

and enteropathy. Time between olmesartan prescription and first symptoms was 28 months [2–139].

Clinical, biological and histological manifestations

All 32 patients (100%) had diarrhoea, with a median number of 8 liquid stools per day [2–20]. Twenty-four patients (75%) had abdominal pain, which was rarely severe (n = 3). Eighteen patients (56%) had vomiting. Body weight loss was 18% [0–48]. Nine patients (28%) had extra-intestinal manifestations, which are described in Table S3. Twenty-three patients (72%) had complications, which are displayed in Table 1. The median duration of symptoms was 10 months [1–53]. Fifteen patients (47%) had anaemia, 28 (88%) had hypokalaemia and 18 (56%) had metabolic acidosis. Twenty-two patients (69%) had acute renal failure. The median serum creatinine level was 188 µmol/L [43–700]. The median serum albumin level was 28 g/L [13–41]. Twenty-three patients (72%) had vitamin or mineral deficiency.

Eleven of 18 patients tested were HLA DQ2 or DQ8 positive. Anti-transglutaminase, anti-endomysium, anti-gliadin and anti-enterocyte antibodies were negative (30/31, 21/21, 11/12 and 13/13 respectively). The patient with positive anti-transglutaminase antibodies (19 IU/mL; upper limit = 7) normalised her antibody levels on further dosages, including those achieved after gluten reintroduction. Anti-nuclear antibodies were positive (9/11) at a median level of 1/1280 [1/320–1/1600].

An upper GI endoscopic description was available for 29 patients (91%) and showed a normal duodenum in 15 cases (52%), an atrophic aspect with mosaic mucosa in 12 cases (41%), ulcerations in four cases (14%). Gastritis was found in two cases (7%). Endoscopic appearance of the jejunum or ileum was described for 17 patients (53%), either by capsule endoscopy or by ileocolonoscopy. Eight patients (47%) had normal small bowels on endoscopy, six patients (35%) had an atrophic aspect and two patients (12%) had jejunal and/or ileal ulcerations. Histological analysis of duodenal biopsies showed villous atrophy in all cases (32/32; 100%), mostly subtotal or total (26/30; 87%). Seventeen of 26 patients (65%) had counts of intra-epithelial lymphocytes of 30/100 epithelial cells or more. There was a lymphocytic infiltrate of the lamina propria (31/32; 97%), crypt hypertrophy (9/26; 35%); and, in two patients, collagen sprue (2/26; 8%). All intra-epithelial lymphocytes were CD3+ (18/18; 100%), CD8+ (14/14; 100%) and were polyclonal as indicated by the lack of detectable clonal T-cell receptor gamma rearrangement in duodenal biopsies (11/11). Lymphocytic colitis was found in four cases. Phenotypic studies of peripheral blood lymphocytes were performed in eight patients during olmesartan treatment and did not show any specific pattern, except for the presence of activated T cells in three of five patients tested (15% CD4+DR+, 45% CD4+CD25+, 52% CD8+DR+).

Table 1 | Complications observed in patients with olmesartan-associated enteropathy

Complications	Patients with villous atrophy, N = 32	Patients with normal villi, N = 4
Dehydration	14	3
Sepsis	5	–
Venous thrombosis	5	–
Cardiac arrhythmia	4	–
Pulmonary oedema	2	–
Pancreatitis	1	–
Rhabdomyolysis	1	–
Hyperosmolar coma	–	1
Ischaemic colitis	1	–
Steroid-associated fracture of the femur	1	–
Total number of patients	23	3

Outcome

No patient died. Thirty-one patients were hospitalised. The median cumulative hospital stay was 29 days [8–460]. Four patients required intensive care (12 days [5–22]). Seven patients required enteral nutrition (30 days [7–100]) and 10 required parenteral nutrition (29 days [10–291]). At the time of diagnosis, olmesartan was not known as a potential cause of enteropathy; therefore most patients were treated as if they had an autoimmune enteropathy. Nine of 14 patients responded to corticosteroids. Some patients received immunosuppressants, either alone or combined with corticosteroids. Four of five, one of two and six of seven patients achieved remission with thiopurines, tacrolimus and anti-TNF respectively. In total, 14 of 15 patients had remission with steroids and/or immunosuppressants. A gluten-free diet (GFD) was introduced in 21 patients and was insufficient in the 13 patients in whom it could be evaluated. Response to the GFD could not be evaluated in eight patients [two with only 3 and 7 days of the GFD and six in whom the GFD was introduced concomitantly with steroids (n = 1) or olmesartan withdrawal (n = 5)].

Twelve patients had 23 olmesartan interruptions followed by reintroductions. All of them were performed

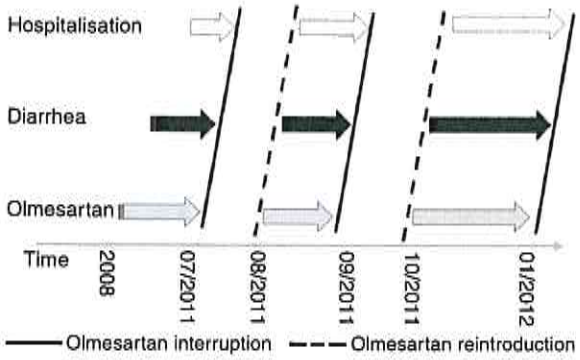

Figure 1 | The effect of interruption and reintroduction of olmesartan in a patient with olmesartan-associated enteropathy. Olmesartan (grey arrow) was stopped because of hypotension due to severe diarrhoea. Interruption of olmesartan led to improvement of diarrhoea (black arrow) and discharge from hospital (white arrow). Olmesartan was started again, which led to relapse of diarrhoea and hospitalisation.

before physicians were aware of the olmesartan-associated enteropathy. In most cases, interruptions were motivated by hypotension and reintroduction was motivated by patient improvement. We studied the 10 olmesartan interruptions and reintroductions performed before initiating treatment with steroids and immunosuppressants, to avoid the effect of these confounders. Olmesartan interruptions were followed by clinical remissions in 9 of 10 cases. Olmesartan reintroductions were followed by clinical relapses in nine of nine cases. An illustrative case is shown on Figure 1.

Exactly, 29/32 patients had been in clinical remission with a median follow-up of 8 months [0–40] since olmesartan interruption, including 26 with no immunosuppressants. Two patients were in remission with olmesartan and immunosuppressants (Infliximab and purinethol for one, azathioprine for the other) and one patient was not in clinical remission, 1 month after olmesartan withdrawal.

Four cases of olmesartan-associated sprue have occurred since the electronic alert in July 2013. None of these patients were prescribed systemic steroids or immunosuppressants and all completely recovered after olmesartan was stopped. In these four patients, the median time from the first symptoms to olmesartan cessation was 3 months [1–4] and the median duration of symptoms was 3 months [2–4]. Comparatively, in the rest of the cohort, the median time from the first symptoms to olmesartan cessation was 9 months [0–68] and the median duration of symptoms was 11 months [1–53]. Fifteen patients with villous atrophy had duodenal biopsies 9 months [2–39] after olmesartan cessation: the biopsy results came back normal for all of them.

Enteropathy with normal villi associated with olmesartan

Four patients (one man and three women, aged 55–74) had enteropathy and no villous atrophy. Three of them had a personal history of autoimmune or inflammatory disease (Table S1). One patient received 10 mg of olmesartan, the others received 40 mg. Three patients received other treatments concomitantly with olmesartan (Table S2).

Symptoms appeared 49 months [0–114] after olmesartan prescription and went on for 10 months [6–24]. All patients had diarrhoea, three had abdominal pain and one had vomiting. Body weight loss was 10% [0–23]. Three patients had hypokalaemia, one had metabolic acidosis, three had severe dehydration and acute renal failure (one patient required dialysis); one patient had hyperosmolar coma (Table 1). Two patients had low serum albumin level, respectively 27 and 29 g/L. None had anti-transglutaminase, anti-gliadin, anti-endomysium or anti-enterocyte antibodies. One patient was DQ2+. Endoscopic duodenal description was available in three patients; it showed an atrophic aspect in one case and duodenal ulcerations in two cases. On histological examination, two patients had elevated intra-epithelial lymphocyte counts (>30/100 epithelial cells) and three patients had lymphocytic infiltrate of the lamina propria. Three patients had microscopic colitis (two collagen colitis, and one lymphocytic colitis).

No patient died. Three patients were hospitalised (12 days [12–22]), and two required intensive care. No patient received artificial nutrition, steroids or immunosuppressants. No patient responded to the gluten-free diet. All patients recovered after olmesartan withdrawal. Two patients had olmesartan reintroduction; one relapsed and eventually achieved remission after the second olmesartan interruption. One patient relapsed 1 month after olmesartan withdrawal and remained steroid-dependent; he had collagen colitis.

Enteropathy associated with irbesartan

A 54-year-old woman has received irbesartan for hypertension since August 2011. From May 2012, she has had abdominal pain, 39% body weight loss, and acute renal failure. Duodenal biopsies showed total villous atrophy. Anti-transglutaminase, anti-endomysium, anti-enterocyte

antibodies and HLA DQ2/DQ8 were negative. Irbesartan was stopped in July 2013. Clinical remission was obtained, parenteral and enteral nutrition was stopped, and the patient was discharged from the hospital.

DISCUSSION

This study confirms the association between olmesartan and a sprue-like enteropathy, which is very similar with that described by Rubio-Tapia *et al.*[15] We also describe patients with severe clinical enteropathies without villous atrophy. Olmesartan is likely to be causal, as interruptions were followed by clinical remissions and reintroductions were followed by relapses. The association seems much more common with olmesartan than with other sartans.

Patients included in this study had a severe course. Some of them had life-threatening diarrhoea with acute renal failure, severe hypokalaemia and metabolic acidosis, leading to prolonged hospitalisation and, sometimes a visit to the ICU. They recovered after olmesartan withdrawal. The most recent cases had a shorter course than the early cases, thanks to the awareness of Gastroenterologists who immediately stopped olmesartan.

This study was performed throughout France, either in private or in public practice, tertiary referral hospitals as well as primary care. However, only gastroenterologists were contacted. This could have led to underestimate the frequency of olmesartan-associated enteropathy and biased the results towards the most severe forms.

This study supports the causality of the association between olmesartan and enteropathy. Firstly, our cases and those reported by Rubio Tapia *et al.* [15] were remarkably similar. Secondly, nondeliberate interruptions followed by reintroductions led to clinical remissions followed by clinical relapses respectively. Thirdly, as in the study by Rubio-Tapia *et al.*, duodenal mucosa returned to normal after olmesartan withdrawal. In addition, a recent epidemiological study performed in France has shown a significant association between hospitalisation for malabsorption and olmesartan prescription as compared with angiotensin-converting enzyme inhibitors and other sartans.[21] The lack of association between gastrointestinal events and olmesartan found in the ROADMAP trial [19] and in the paper by Padwal *et al.* [20] could be due to the confounding effect of diabetic neuropathy, which may provoke chronic diarrhoea. In addition, the incidence of olmesartan enteropathy is low and may require large populations of patients to be clearly demonstrated.

We report four patients with olmesartan-associated enteropathy and normal villi. The clinical picture was that of severe diarrhoea, similar with that of patients with villous atrophy, and these four patients also improved after olmesartan withdrawal. These cases add to the description of olmesartan-associated enteropathy. It may include patients with a wide range of histological duodenal abnormalities, from isolated intra-epithelial lymphocytosis and lamina propria lymphocytic infiltration to total villous atrophy. In addition, there is evidence of involvement of almost the entire gut in this condition.[15, 22] Biopsies of the duodenum but also of the stomach and the colon should be performed in patients with suspected olmesartan-associated enteropathy.

Eleven of 18 patients with villous atrophy tested (61%) were either DQ2+ or DQ8+, the coeliac disease predisposing phenotype. However, coeliac disease serology was negative and patients did not respond to a gluten-free diet, meaning that olmesartan does not trigger coeliac disease, as observed with interferon [23] and ipilimumab.[24] Rather, olmesartan-associated enteropathy appears as a separate immune-mediated entity. Firstly, a past history of autoimmunity or inflammatory disorders is frequent. Secondly, it may be associated with anti-nuclear antibodies and circulating activated T cells. Thirdly, all patients tested had polyclonal CD3+ CD8+ intra-epithelial lymphocytosis, which could mimic type 1 refractory coeliac sprue. Finally, it appears to respond to steroids or immunosuppressants and/or anti-TNF monoclonal antibodies. However, olmesartan-associated enteropathy differs from autoimmune enteropathy because it is associated with anti-nuclear antibodies and not with anti-enterocyte antibodies. A lupus-associated protein-losing enteropathy has been described.[25] But it had been reported long before olmesartan was marketed. The clinical and pathological picture is very different from that of olmesartan-associated enteropathy. It typically occurs in young women. The main symptom is generalised oedema due to profound hypoalbuminaemia; diarrhoea is observed in only half of the cases. Eventually, small bowel endoscopy and biopsies are normal in a lupus-associated protein-losing enteropathy.

In conclusion, this study shows that olmesartan causes severe and potentially life-threatening enteropathy with or without villous atrophy. It appears to be a new disease that differs from other immune-mediated enteropathies, such as coeliac disease, lupus-associated protein-losing enteropathies and autoimmune enteropathies. The pathophysiology of olmesartan-associated enteropathies requires further investigation that may shed new light on

coeliac disease and intestinal auto immunity. Patients who receive and physicians who prescribe olmesartan should be advised to stop the drug if diarrhoea appears.

AUTHORSHIP

Guarantor of the article: Prof. Franck Carbonnel.

Author contributions: Marthey L, Cadiot G, Seksik P, Pouderoux P, Lacroute J, Skinazi F, Mesnard B, Chayvialle JA, Savoye G, Druez A, Parlier D, Abitbol V, Gompel M, Eoche M, Poncin E, Bobichon R, Colardelle P, Wils P, Salloum H, Peschard S, Zerbib F, Malamut G, Carbonnel F: Acquisition of data. Marthey L, Cadiot G, Méresse B, Cerf-Bensussan N, Carbonnel F: Analysis of data, drafting and critical revision of the manuscript. All authors approved the final version of the manuscript.

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Declaration of personal interests: Marthey L has served as a speaker for Amgen Cadiot G declares no conflict of interest Seksik P has served as a consultant for Abbvie, MSD, Servier, and Biocodex, Pouderoux P, Skinazi F, Mesnard B, Druez A, Parlier D, Peschard S, Méresse B, Cerf-Bensussan N, Malamut G, Salloum H, Wils P, Gompel M, Lacroute J, Chayvialle JA, Eoche M, Poncin E, Bobichon R and Collardelle P declare no conflict of interest Savoye G has served as a speaker for MSD, Abbvie, Ferring, Vifor, HAC Pharma and Pharmacosmos Abitbol V has served as a speaker for Abbvie, MSD, Ferring, Vifor Zerbib F has served as a consultant and board member for Given Imaging, Addex Pharma, Shire Movetis, Almirall, Reckitt Benckiser and Mederi Therapeutics; and as a speaker for Abbvie, Coloplast and Mayoli Spindler Carbonnel F has served as an advisory board member for Otsuka and Genentech.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Patients with olmesartan-associated enteropathy presenting past history of autoimmune or inflammatory disease.

Table S2. drugs prescribed concomitantly with olmesartan in at least two patients in the olmesartan-associated enteropathy cohort.

Table S3. extra-intestinal manifestations in patients with olmesartan-associated enteropathy.

REFERENCES

1. Pallav K, Leffler DA, Tariq S, *et al.* Non coeliac enteropathy: the differential diagnosis of villous atrophy in contemporary clinical practice. *Aliment Pharmacol Ther* 2012; **35**: 380–90.
2. Ghoshal UC, Srivastava D, Verma A, Ghoshal U. Tropical sprue in 2014: the new face of an old disease. *Curr Gastroenterol Rep* 2014; **16**: 391.
3. Carbonnel F, Grollet-Bioul L, Brouet JC, *et al.* Are complicated forms of celiac disease cryptic T-cell lymphomas? *Blood* 1998; **92**: 3879–86.
4. Cellier C, Delabesse E, Helmer C, *et al.* ; French Coeliac Disease Study Group. Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. *Lancet* 2000; **356**: 203–8.
5. Rubio-Tapia A, Kelly DG, Lahr BD, Dogan A, Wu TT, Murray JA. Clinical staging and survival in refractory celiac disease: a single center experience. *Gastroenterology* 2009; **136**: 99–107.
6. Daum S, Weiss D, Hummel M, *et al.* ; Intestinal Lymphoma Study Group. Frequency of clonal intraepithelial T lymphocyte proliferations in enteropathy-type intestinal T cell lymphoma, coeliac disease, and refractory sprue. *Gut* 2001; **49**: 804–12.
7. Farstad IN, Johansen FE, Vlatkovic L, *et al.* Heterogeneity of intraepithelial lymphocytes in refractory sprue: potential implications of CD30 expression. *Gut* 2002; **51**: 372–8.
8. Verbeek WH, Goerres MS, Von Blomberg BM, *et al.* Flow cytometric determination of aberrant intra-epithelial lymphocytes predicts T-cell lymphoma development more accurately than T-cell clonality analysis in Refractory Celiac Disease. *Clin Immunol* 2008; **126**: 48–56.
9. Culliford AN, Green PH. Refractory sprue. *Curr Gastroenterol Rep* 2003; **5**: 373–8.
10. Carbonnel F, d'Almagne H, Lavergne A, *et al.* The clinicopathological features of extensive small intestinal CD4 T cell infiltration. *Gut* 1999; **45**: 662–7.
11. Malamut G, Meresse B, Kaltenbach S, *et al.* Small intestinal CD4+ T-Cell lymphoma is a heterogenous entity with common pathology features. *Clin Gastroenterol Hepatol* 2014; **12**: 599–608.
12. Margolskee E, Jobanputra V, Lewis SK, Alobeid B, Green PH, Bhagat G. Indolent small intestinal CD4+ T-cell lymphoma is a distinct entity with unique biologic and clinical features. *PLoS ONE* 2013; **8**: e68343.
13. Corazza GR, Biagi F, Volta U, Andreani ML, De Franceschi L, Gasbarrini G. Autoimmune enteropathy and villous atrophy in adults. *Lancet* 1997; **350**: 106–9.
14. Akram S, Murray JA, Pardi DS, *et al.* Adult autoimmune enteropathy: Mayo Clinic Rochester experience. *Clin Gastroenterol Hepatol* 2007; **5**: 1282–90.
15. Rubio-Tapia A, Herman ML, Ludvigsson JF, *et al.* Severe sprue-like

enteropathy associated with Olmesartan. *Mayo Clin Proc* 2012; **87**: 732.
16. De Gaetani M, Tennyson CA, Lebwohl B, *et al.* Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma. *Am J Gastroenterol* 2013; **108**: 647–53.
17. Ianiro G, Bibbò S, Montalto M, Ricci R, Gasbarrini A, Cammarota G. Systematic review: sprue-like enteropathy associated with olmesartan. *Aliment Pharmacol Ther* 2014; **40**: 16–23.
18. Haller H, Ito S, Izzo JL, *et al.* Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. *N Engl J Med* 2011; **364**: 907–17.
19. Menne J, Haller H. Olmesartan and intestinal adverse effects in the ROADMAP study. *Mayo Clin Proc* 2012; **87**: 1230.
20. Padwal R, Lin M, Etminan M, Eurich DT. Comparative effectiveness of olmesartan and other Angiotensin receptor blockers in diabetes mellitus: retrospective cohort study. *Hypertension* 2014; **63**: 977–83.
21. Basson M, Mezzarobba M, Weill A, *et al.* Severe malabsorption associated with olmesartan: a French Nationwide Cohort Study. *Gastroenterology* 2014; **146**: S114 (abstract).
22. Fiorucci G, Puxeddu E, Colella R, Paolo Reboldi G, Villanacci V, Bassotti G. Severe spruelike enteropathy due to olmesartan. *Rev Esp Enferm Dig* 2014; **106**: 142–4.
23. Fernandez-Salazar L, Alvarez-Quinones M, Gonzalez Hernandez JM, *et al.* IFN beta 1b induced celiac disease. *Scand J Gastroenterol* 2011; **46**: 1525–6.
24. Gentile NM, D'Souza A, Fujii LL, Wu TT, Murray JA. Association between ipilimumab and celiac disease. *Mayo Clin Proc* 2013; **88**: 414–7.
25. Perednia DA, Curosh NA. Lupus-associated protein-losing enteropathy. *Arch Intern Med* 1990; **150**: 1806–10.

EXHIBIT 6

Etiology

Use of olmesartan for ≥ 1 year was associated with hospitalization for intestinal malabsorption

Basson M, Mezzarobba M, Weill A, et al. ***Severe intestinal malabsorption associated with olmesartan: a French nationwide observational cohort study.*** *Gut. 2015 Aug 6. [Epub ahead of print]*

Clinical impact ratings: GIM ★★★★★★☆ GI ★★★★★★★ G ★★★★★★☆

Question

Is use of olmesartan associated with hospitalization for intestinal malabsorption?

Methods

Design: Cohort study with linkage of national databases.

Setting: France.

Patients: 4 546 680 patients (mean age 61 to 64 y across treatment groups, 46% to 56% women across treatment groups) who initiated treatment with an angiotensin-receptor blocker (ARB) or angiotensin-converting enzyme (ACE) inhibitor. Exclusion criteria were enrollment in the Système National d'Information Interrégimes de l'Assurance Maladie database for < 1 year before the first filled ARB or ACE inhibitor prescription (index date); absence of prescription claims 1 to 2 years before the index date; or, in the year before the index date, filled prescriptions for ARBs, ACE inhibitors, or gluten-free diet products, hospitalization for intestinal malabsorption, or celiac disease-specific serologic testing.

Risk factors: Filled prescription for olmesartan, other ARBs, or ACE inhibitors (exposure period was from the date the prescription was filled until 30 d after the end of prescription duration).

Outcomes: Hospitalization with discharge diagnosis of intestinal malabsorption. Secondary outcome was hospitalization with a discharge diagnosis of celiac disease.

Main results

Use of olmesartan, but not other ARBs, was associated with risk for hospitalization for intestinal malabsorption (Table). Use of olmesartan overall (adjusted rate ratio [aRR] 4.39, 95% CI 2.77 to 6.96), for 1 to 2 years (aRR 4.36, CI 2.04 to 9.34), or for > 2 years (aRR 10.21, CI 4.21 to 24.76) was associated with hospitalization for celiac disease; use for < 1 year (aRR 1.98, CI 0.85 to 4.61) was not. Use of other ARBs was not associated with hospitalization for celiac disease overall or at any time interval (all $P \geq 0.51$).

Conclusion

Use of olmesartan for ≥ 1 year was associated with hospitalization for intestinal malabsorption.

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For correspondence: Dr. F. Carbonnel, CHU de Bicêtre, Le Kremlin Bicêtre, France. E-mail fcarbonnel7@gmail.com.

Association between olmesartan or other angiotensin-receptor blockers and hospitalization for intestinal malabsorption*

Treatment duration	*Adjusted rate ratio (95% CI)†*	
	Olmesartan	*Other angiotensin-receptor blockers*
All	2.49 (1.73 to 3.57)	0.78 (0.58 to 1.07)
< 1 y	0.76 (0.39 to 1.49)	0.58 (0.38 to 0.88)
1 to 2 y	3.66 (1.84 to 7.29)	1.03 (0.56 to 1.92)
> 2 y	10.65 (5.05 to 22.46)	1.68 (0.80 to 3.51)

*CI defined in Glossary.

†Compared with use of angiotensin-converting enzyme inhibitors. Adjusted for several demographic and clinical variables.

Commentary

The well-conducted database study by Basson and colleagues puts to bed any controversy surrounding the association between the ARB olmesartan and severe intestinal enteropathy pathologically resembling celiac disease. Surrogate diagnoses for olmesartan enteropathy were used to search the database, selection bias was minimized, and confounding was carefully considered.

Evidence supporting a causal relation now includes the strength of association, consistent findings, evidence of improvement in most patients after discontinuation, and relapse on drug reintroduction (1-3). The mechanism remains to be established but, based on comparative data, the effect seems to be drug-specific rather than class-specific. Olmesartan is a pro-drug that, in the gut mucosa, is quickly metabolized to its active form. The long gap between drug exposure and disease makes type I hypersensitivity unlikely (2), and evidence implicates an immunopathologic pathway similar to gluten intolerance (4).

Other important, albeit unusual, causes of subtotal villous atrophy on duodenal biopsy in the absence of elevated tissue transglutaminase levels include autoimmune enteropathy and common variable immune deficiency. Olmesartan enteropathy can overlap with small bowel bacterial overgrowth, another cause of diarrhea, but the former does not respond to antibiotics (2).

The number needed to harm is admittedly large (after 2 y, 12 500 patient-y of exposure to cause 1 additional case of enteropathy). However, the study provides the incidence of the most severe forms of olmesartan-associated enteropathy and may underestimate the incidence of enteropathy of all severities. All patients should be warned to advise their physician and stop the drug if they develop any diarrhea or weight loss on olmesartan.

Nicholas J. Talley, MD, PhD
University of Newcastle
Callaghan, New South Wales, Australia

References

1. **Rubio-Tapia A, Talley NJ, Gurudu SR, Wu TT, Murray JA.** Gluten-free diet and steroid treatment are effective therapy for most patients with collagenous sprue. Clin Gastroenterol Hepatol. 2010;8:344-9.e3.
2. **Rubio-Tapia A, Herman ML, Ludvigsson JF, et al.** Severe spruelike enteropathy associated with olmesartan. Mayo Clin Proc. 2012;87:732-8.
3. **Marthey L, Cadiot G, Seksik P, et al.** Olmesartan-associated enteropathy: results of a national survey. Aliment Pharmacol Ther. 2014;40:1103-9.
4. **Marietta EV, Nadeau AM, Cartee AK, et al.** Immunopathogenesis of olmesartan-associated enteropathy. Aliment Pharmacol Ther. 2015;42:1303-14.

